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Paddy rice stored under hermetic conditions: The effect of relative humidity, temperature and storage time in suppressing Sitophilus zeamais and impact on rice quality

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A B S T R A C T

The aim of this study was to analyse the effect of relative humidity in suppressing Sitophilus zeamais, in paddy rice stored under hermetic conditions, during four and seven months, at different average temperatures, as well as the impact on rice quality.

Hermetic bags, GrainPro® SuperGrainbag® Farm™, were used to store two rice varieties under three different relative humidities: 67%, 75% and 85% RH, and average temperatures of 14 °C, 17 °C and 24 °C, both monitored by Hobo® Data loggers, with the probe placed inside the bags. CheckpointII Portable O_2 and CO_2 Gas Analyzer was used to assess gas contents on the top and bottom of each bag. At the end of the trials, paddy samples were collected to estimate water activity (a_w). The rheology behaviour of rice pastes prepared with race flour obtained from the different treatments was also evaluated, using a controlled stress rheometer.

The results showed that the response of the stored-product insects changes with environmental conditions, O_2 and CO_2 contents. Other parameters were considered; a_w increased with relative humidity and temperature, but decreased with storage time. The relative humidity played an important role, together with the increase of temperature, in suppressing insect populations. A modified atmosphere was naturally produced inside the hermetic bag, under 85% RH, with low O_2 and high CO_2 contents, at different average temperatures, 14 °C and 17 °C. These results demonstrated that *S. zeamais* can survive, but has no progeny. Under the same conditions, but at the higher average temperature of 24 °C, *S. zeamais* attained 100% mortality before producing progeny.

The increase on respiration rate, registered by CO_2 increase and O_2 decrease, for higher RH values, reduced the viscoelastic functions and changed the starch gelatinization point of Indica and Japonica rice.

The results obtained showed that storing paddy hermetically, at low relative humidity, did not change atmospheric content and maintained the viscoelastic functions of the rice pastes.

1. Introduction

Humans have known, for at least 2500 years that hermetic storage can be used for the preservation of dry products (Adler et al., 2000). The principle of hermetic storage is to avoid any interactions with the surrounding environment. This is considered as a type of modified atmospheres (MA), because this method takes the advantage of the gases naturally produced by the respiratory metabolism of the insects, fungi and commodity itself, generating a MA by reducing O_2 and increasing CO_2 concentrations, and consequently preventing insects and fungi development (Navarro, 2006). The storage life of food products is considerably extended by MA surrounding the food, which reduces also the respiration rate of the food products. (Jayas and Jeyamkondan, 2002). This is one of the natural food preservation methods that maintains the quality of food products in addition to extending the storage life.

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This method has been extensively used on grain namely on rice preservation (Navarro, 1978; Moreno-Martinez et al., 2000; Jayas and Jeyamkondan, 2002; Conyers and Bell, 2007). The exposure time needed to control the insect populations and to protect the grain depends on the degree of infestation, environmental conditions and storage time. The rate of atmosphere modification also depends on environmental conditions and degree of infestation.

Portugal is the largest consumer of rice per capita (16 kg per capita) in Europe (average 8 kg), and consequently a large number of farmers and industries are associated with rice production, transportation, storage and processing. In Portugal, rice is a seasonal crop and storage is very important for year round availability. Planting of rice takes place in April and harvest is near the end of August (Carvalho et al., 2012b). After harvesting, drying is the most critical operation. Delays on drying, incomplete drying or ineffective drying might reduce grain quality and result in considerable economic losses (Sinha et al., 2010). The flux of the grain can surpass the drying capacity resulting in difficulties to dry the cereal properly. In some regions of Portugal, rice is stored as paddy on farm horizontal warehouses or vertical silos, until the end of winter. The major pest of stored rice in Portugal is the maize weevil, *Sitophilus zeamais* Motschulsky. Applications of hermetic storage and modified atmospheres to preserve the quality and flavour of rice during storage can be promising, safe and environmental friendly.

The aim of this study was to evaluate the relationship between hermetic storage at three different relative humidities, under different storage times (four months and seven months), temperature average and water activity, and the influence of hermetic storage on *S. zeamais* populations, and rice quality. The rice quality was evaluated based on the rheology behavior of rice aqueous pastes (Torres et al., 2013) from rice flour to detect structural changes at the macromolecule (mainly starch) level.

2. Material and methods

2.1. Sample preparation

Experiments were conducted in a warehouse, to store paddy, property of the rice farmers association of Sado Valley region, Portugal. Three trials were carried out from December to November: [T_1] the first trial, four months, from December to April, corresponds to the mean storage time of paddy in this Association, before being delivered to the rice mills; [T_2] the second trial from December to July, seven months of storage; [T_3], the third trial, four months, from July to November, with higher average temperatures.

For all the experiments, GrainPro® SuperGrainbag® Farm™ bags were used to store two rice varieties: Ronaldo, *Oryza sativa* sbsp. japonica, (developed by Lugano Leonardo©, Italy), and Sírio, *Oryza sativa* sbsp. indica (developed by SA.PI.SE., Italy). Both varieties are the most used by the farmers in 2015, 2016 and 2017, in Sado Valley. Same varieties were stored in jute bags, as the control. Both jute bags and SuperGrainbag® have a capacity of 50 kg, and for each treatment and variety three replications were carried out. A total of 48 samples per trial were evaluated. The relative humidity and temperature were monitored by Hobo® Data loggers, with the probe placed inside the bags.

In all experiments, the two varieties were stored as paddy and submitted to three different relative humidities: 67%, 75% and 85% RH, at three different average temperatures: 14 °C, 17 °C and 24 °C. The temperature recorded by the several data loggers are identical in all bags for the same trial, regardless of the humidity and type of rice. However the temperature varied during the storage period (Fig. 1). T_1 had the temperature average of 14 °C with no record above 20 °C; T_2 had an average temperature of 17 °C with 43 days above 20 °C and 13 days above 25 °C; T_3 had the highest mean temperature, 24 °C, with 103 days above 20 °C, in which 60 days were over 25 °C and 34 days above 27 °C (Table 1). The December to July trial [T_2] was much longer than the other two experiments, while the highest temperatures occurred in the July to November trial (Fig. 1).

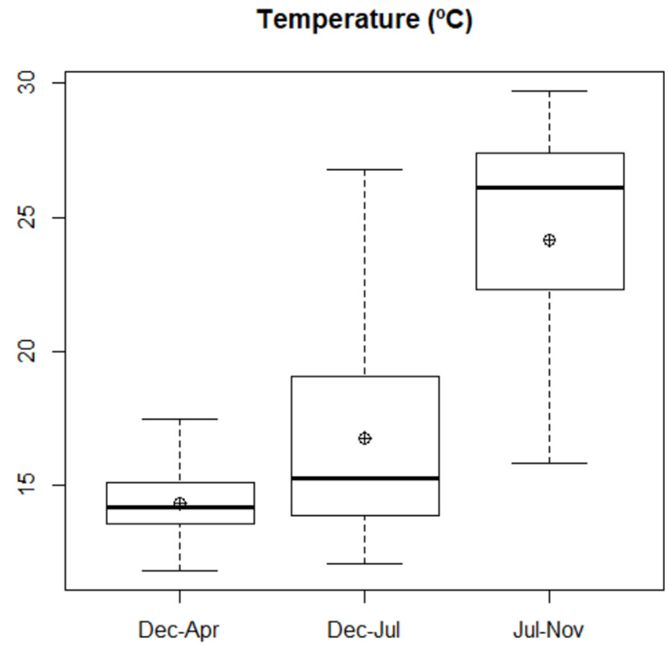

Fig. 1. Distribution of recorded temperature in each experiment. The thicker line represents the median (half of the records lies above the line) while the symbol represents the mean temperature across the entire experiment.

At the end of the four (T_1 and T_3) and seven (T_2) months of experiments, all bags were opened and paddy samples were collected. Before opening the bags, CheckpointII Portable O_2 and CO_2 Gas Analyzer was used to assess gas contents, at the bottom and top of each bag, making for a total of six measures per treatment, and the results registered. The gas content is expressed in terms of percentage by volume in air. Samples were taken to be dehusked and milled to analyse water activity (a_w), with three replications per treatment. Rice pastes were prepared to perform the rheology tests.

HygroPalm HP23 Rotronic was used to estimate $a_{w,}$ using three replications per treatment.

2.2. Bioassays

To evaluate the insects' response to each treatment, *S. zeamais* was chosen since it is the key pest of rice in Portugal (Carvalho et al., 2012a). *S. zeamais* was originally collected from Portuguese rice mills and reared in climatic chambers, at 25±2 °C and 70 ± 2% RH, at our laboratory.

Table 1
Duration of each experiment in days, mean temperature and number of "hot days". 'number days' is the number of days with mean temperature greater then 20 °C, 25 °C and 27 °C.

	[T_1]	[T_2]	[T_3]
Storage period (days)	139	215	121
Mean Temperature (°C)	14 ± 1	17 ± 4	24 ± 3
number days with Temp>20 °C	0	43	103
number days with Temp>25 °C	0	13	60
number days with Temp>27 °C	0	0	34

For experiments, 20g of brown rice were infested with one-week-old of ~20 *S. zeamais* adults and placed inside paper bags. One paper bag was settled up inside of each paddy bag, totalizing three replications per treatment.

2.3. Rheology measurements

Rheology tests were carried out for T_1 trial and performed on a MARS III controlled-stress rheometer (Haake) coupled with a temperature controlling Peltier unit, using a 35 mm diameter serrated parallel plates and 0.5 mm gap. Aqueous flour suspensions (10% w/w) were held 5 min at 20 °C, between the plates, before testing. Considering the following steps: (a) Heating curves from 20 °C to 90 °C (2 °C/min), oscillatory frequency of 0.1Hz; (b) Setting at 90 °C during 1 h; (c) Colling curves from 90 °C to 5 °C (-1 °C/min); (d) Maturation curve during 1 h at 5 °C; (e) Mechanical spectra with frequency range between 0.01 and 100 Hz at 20 °C. Then, the rheological study using SAOS (small amplitude oscillatory system) was performed according to previously optimized conditions (Torres et al., 2014).

2.4. Data analysis

All the computations and graphics were performed with the R software (R Core Team, 2017). Function *lm* was used to fit and test for significance all the linear models. The simple linear regressions of CO_2 vs O_2 were fitted to 9 data points; for each experimental condition of temperature, relative humidity and storage time, the average of all gas measurements was used.

Linear models were also considered aiming at to relate the atmospheric composition at the end of each assay (oxygen content, carbon dioxide content and water activity) with the temperature, relative humidity and the trial duration. Each trial (T1 = 14 °C, 4months; T2 = 17 °C, 7months and T3 = 12 °C, 4months) was carried out at three humidity conditions (RH = 67%, 75% and 85%). For each of these 9 experimental conditions all the individual measurements of O_2 (6), CO_2 (6) and a_w (3–10) were used to fit the linear models. These replications are based on independent samples.

Beside these linear models, a 2-way ANOVA with interaction model was fitted to the O_2 and a_w data using as factors: the combination temperature/time (Temp_time, with 3 levels: 14_4, 17_7 and 24_4) and the relative humidity (RH, with 3 levels: 67, 75 and 85). The data collected for the two rice varieties were merged. For each of the 9 cells, two replications were used, each one corresponding to the average of the measurements for a rice variety. Thus, 18 observations were used to fit each 2-way ANOVA.

The same two-factor factorial design was used to analyse the relation between insect's growth and storage conditions. A 2-way ANOVA with interaction model was fitted to the progeny observed in each of the four to seven small bags containing rice initially contaminated with 20 insects. Globally 54 observations were used to fit the model.

The ANOVA models were fitted with the R function *aov*. The post hoc Tukey and Kruskal-Wallis with Fisher's least significant difference tests were performed with functions *HSD.test* and *kruskal* from package 'agricolae' (Mendiburu, 2017).

3. Results

3.1. Gas content and water activity (a_w)

The change in air composition was assessed by measuring the oxygen and carbon dioxide inside each bag at the end of each experiment. The carbon dioxide is linearly and negatively related to the oxygen content (Fig. 2). The straight lines are very precise (both R^2 are close to 1) and similar for Indica and Japonica rice. In average, when the oxygen increases 1%, the carbon dioxide decreases 1.16% for Indica and 1.29% for Japonica. When all the oxygen was consumed ($O_2 \approx 0\%$), the CO_2 produced is slightly higher at Japonica rice than at Indica rice (Fig. 2).

After four months (T_1 and T_3) and seven months (T_2) at different average temperatures, under 67% RH, there was no significant change in O_2 and CO_2 content. Therefore no respiration was detected, which is a sign of no biological activity. At higher relative humidity, there were changes on the gas content, indicating an increase on the respiration rate given by the significant decrease on O_2 and increase on CO_2 with the increase in RH (Fig. 3ab).

The change in air composition was complemented by evaluating water activity (a_w) inside each bag at the end of experiment. The water activity (a_w) at the end of the experiment, for each rice type (Indica and Japonica) showed a dependency on the environmental conditions and storage time (Fig. 4ab).

Linear models were considered aiming at to relate the atmospheric composition at the end of each assay (oxygen content, carbon dioxide content and water activity) with the temperature, relative humidity and the trial duration. For each parameter, two tested models were considered and compared through a partial F test: a complete model allowing a fit for each rice variety and a sub model that does not distinguish the rice varieties. According to the fitted linear models (all considered significant by the global F tests), the increase in temperature (keeping relative humidity and time unchanged) leads to a decrease in oxygen and an increase in carbon dioxide and water activity. Similarly, the increase in relative humidity (keeping the temperature and time constant) leads to a decrease in oxygen and an increase in carbon dioxide and water activity. The storage time (at constant temperature and humidity) has an effect of increasing the carbon dioxide and decreasing oxygen and water activity (Table 2).

The atmosphere composition (O_2 and CO_2) depends in a similar way on the storage conditions (temperature, relative humidity and time) for both rice varieties, since the p-values of the partial F tests

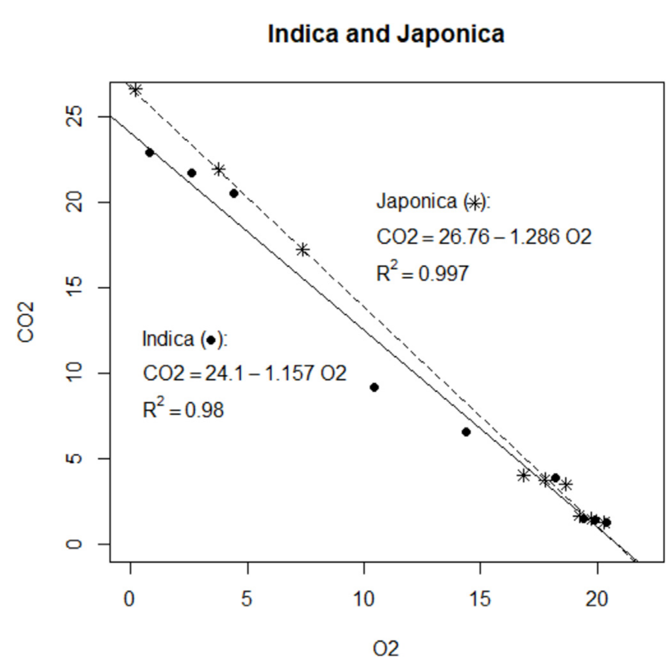

Fig. 2. Relation between the average values of carbon dioxide (CO_2%) and oxygen (O_2%), measured at the end of each experiment.

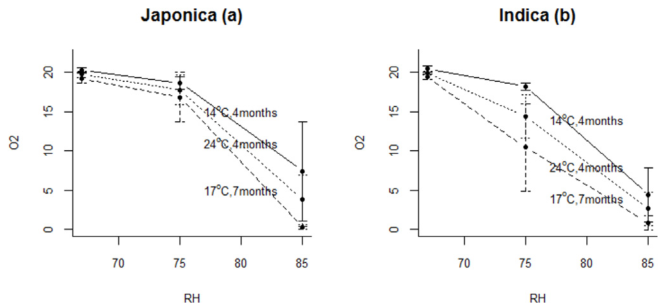

Fig. 3. (a) - Amount of O_2 (%) at the end of the experiment, for Japonica rice and each environmental storage condition (Temperature in °C, time in months and RH in %). The vertical bars indicate the standard deviation of the replications. (b)- Amount of O_2 (%) at the end of the experiment, for Indica rice and each environmental storage condition (Temperature in °C, time in months and RH in %). The vertical bars indicate the standard deviation of the replications.

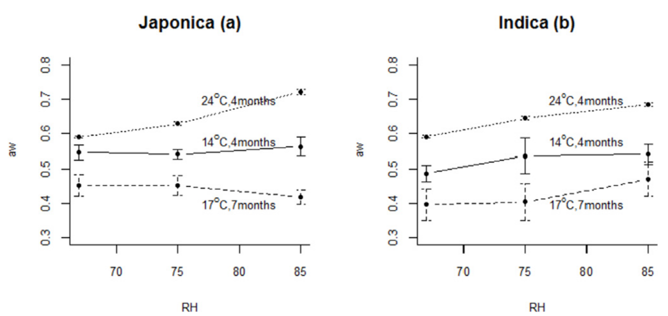

Fig. 4. (a) - Water activity (a_w) at the end of the experiment, for Japonica rice and each environmental storage condition (Temperature in °C, time in months and RH in %). The vertical bars indicate the standard deviation of the replications. (b) - Water activity (a_w) at the end of the experiment, for Indica rice and each environmental storage condition (Temperature in °C, time in months and RH in %). The vertical bars indicate the standard deviation of the replications.

comparing the unique model for both rice varieties with a model for each rice variety are greater than 0.05 (Table 2).

The fitted 2-way ANOVA model shows that the factors Temp_time and RH affect significantly (p-values<0.05) the oxygen content and water activity in the hermetic bag's atmosphere, while the interaction effect was not significant.

The results obtained by a post-hoc Tukey HSD test show no significant differences in mean O_2 content for all the trials under 67% and 75% RH. However, a significant decrease on O_2 content occurs during experiments under 85% RH. Although not significant, at same RH, a decrease on O_2 is observed with the increase of storage time and of average temperature, for the 4 months trials (Table 3).

Regarding a_w, the water activity increases with the increase of temperature and RH, for the same storage time, and decreases with the increase of storage time, although the differences are not always significant (Table 3).

3.2. *Sitophilus zeamais survival at different environmental conditions and atmospheres*

Even if the population growth of *S. zeamais versus* storage environmental conditions did not fit any linear model tested, there are patterns of note in the obtained data (Tables 4 and 5). When the total of insects were considered, the first 20 weevils adults used per replication were subtracted from the sum of alive and dead insects, to better understand if there was progeny during the trials.

Some differences on insects' growth were observed when comparing the obtained results for the two rice varieties: experiments with Indica rice showed that, at oxygen content below 4.4% there was almost no progeny, whatever the average temperature of the trials (Table 4). Experiments with Japonica rice no progeny was achieved under 3.8% of O_2 content. Contradictory results were obtained, where a progeny of 5.3 adults was recorded at oxygen content of 0.2% (Table 5).

Since the MA at Indica and Japonica rice seems similar (Table 2), the results were merged in order to globally appreciate the relation between progeny and the storage conditions (Fig. 5). We applied a 2-way ANOVA for progeny using the factors Temp_time (3 levels: 14_4, 17_7 and 24_4) and RH (3 levels: 67, 75 and 85). The data for all the paper bags were used. The principal effects of both factors and the interaction effect were significant (p-values<0.001). In order to analyse which storage conditions produce significant differences in progeny, the means and the ranks of the number of adults per vial were compared through a post-hoc Tukey HSD test and a Kruskal-Wallis test with the Fisher's least significant difference method (Table 6).

The highest population growth occurs under 24 °C and 67% RH (Table 6, Fig. 5), storage conditions where atmosphere content remained almost unchanged (Table 3). On the other hand, the highest mortality occurs under 85% RH (Table 6, Fig. 5), where the oxygen content in the storage atmosphere has a significant decrease (Table 3).

3.3. *Rheological measurements*

Rheological tests were carried out for T_1 experiments, from December to April, to evaluate the impact of relative humidity even

Table 2
Fitted linear models relating the composition (in %) of the atmosphere inside the bags at the end of the experiment (O_2, CO_2 and a_w) with the mean temperature and relative humidity of the experiment. For each pair (Temp, RH) all the measures of the gases where used in the model. Partial F test compares the unique model for both rice varieties with the complete model that distinguishes the rice varieties.

Fitted Models	R^2	p-value (global F test)	p-value (partial F test)
Indica			
$O_2 = 95.62 - 0.209$ Temp - 0.971 RH - 1.200 time	0.878	<0.01	
$CO_2 = -83.35 + 0.134$ Temp + 1.149 RH + 0.766 time	0.872	<0.01	
$a_w = 0.193 + 0.0120$ Temp + 0.00437 RH - 0.0433 time	0.817	<0.01	
Japonica			
$O_2 = 90.59 - 0.170$ Temp - 0.909 RH - 0.972 time	0.782	<0.01	
$CO_2 = -87.50 + 0.175$ Temp + 1.167 RH + 1.00 time	0.785	<0.01	
$a_w = 0.520 + 0.00993$ Temp + 0.000849 RH - 0.0442 time	0.852	<0.01	
Indica + Japonica			
$O_2 = 93.10 - 0.190$ Temp - 0.940 RH - 1.086 time	0.822	<0.01	0.858
$CO_2 = -85.42 + 0.155$ Temp + 1.158 RH + 0.883 time	0.824	<0.01	0.199
$a_w = 0.350 + 0.0111$ Temp + 0.00263 RH - 0.0431 time	0.808	<0.01	0.017

Table 3
Comparison of average O_2 content and average a_w in the atmosphere at the end of the experiment, across storage environmental conditions. Results were obtained with a post-hoc Tukey HSD test. The critical value of the Studentized Range based on a Tukey's distribution with parameters 9 and 9, at 5% significance, is 5.595. The Minimum Significant Difference is 7.420 for O_2 and 0.111 for a_w. Treatments with the same letter are not significantly different at a 5% significance.

O_2			a_w		
Temp (°C)_ time (months)	RH (%)	O_2 (%)	Temp (°C)_ time (months)	RH(%)	a_w
14_4	67	20.37[a]	24_4	85	0.703[a]
24_4	67	19.85[a]	24_4	75	0.638[ab]
17_7	67	19.33[a]	24_4	67	0.591[bc]
14_4	75	18.43[a]	14_4	85	0.552[bcd]
24_4	75	16.06[a]	14_4	75	0.538[bcd]
17_7	75	13.65[a]	14_4	67	0.516[cde]
14_4	85	5.88[b]	17_7	85	0.444[de]
24_4	85	3.19[b]	17_7	75	0.427[e]
17_7	85	0.50[b]	17_7	67	0.424[e]

Table 4
Mean and standard error for the number of *Sitophilus zeamais* adults: alive adults, dead adults and total (Progeny = alive adults + dead adults −20) for each pair of temperature and relative humidity conditions in Indica. The shown values correspond to averages across the replications, small bags containing rice initially contaminated with 20 insects. The amounts of oxygen (O_2, %), carbon dioxide (CO_2, %) and water activity (a_w) at the end of each experiment are also shown.

Temp (°C) _time (months)		RH (%)	CO_2 (%)	O_2 (%)	a_w	Alive adults mean ± SE	Dead adults mean ± SE	Progeny mean ± SE
T_1	14_4	85	20.6	4.4	0.541	10.3 ± 5.4	7.7 ± 5.4	0[a]
	14_4	75	3.9	18.3	0.536	13.7 ± 1.5	37.3 ± 6.9	31 ± 8.4
	14_4	67	1.2	20.4	0.486	4.3 ± 1.5	44.5 ± 8.1	28.8 ± 8.4
T_2	24_4	85	21.8	2.6	0.685	0.0 ± 0.0	20.0 ± 0.0	0.0 ± 0.0
	24_4	75	6.5	14.4	0.646	86.3 ± 29.6	64.0 ± 16.6	130.3 ± 45.9
	24_4	67	1.4	19.9	0.591	84.0 ± 14.7	93.3 ± 26.7	157.3 ± 40.9
T_3	17_7	85	23	0.8	0.470	0.7 ± 0.7	20.3 ± 0.9	1.0 ± 1.5
	17_7	75	9.2	10.5	0.403	24.5 ± 16.5	46.0 ± 6.0	50.5 ± 22.5
	17_7	67	1.5	19.4	0.396	8.5 ± 4.9	33.8 ± 9.6	22.3 ± 13.9

[a] At the end less than 20 insects were found.

Table 5
Mean and standard error for the number of *Sitophilus zeamais* adults: alive adults, dead adults and total (Progeny = alive adults + dead adults −20) for each pair of temperature and relative humidity conditions in Japonica. The shown values correspond to averages across the replications, small bags containing, initially, contaminated rice with 20 insects. The amounts of oxygen (O_2, %), carbon dioxide (CO_2, %) and water activity content (a_w) at the end of each experiment are also shown.

Temp (°C)_time (months)		RH (%)	CO_2 (%)	O_2 (%)	a_w	Alive adults mean ± SE	Dead adults mean ± SE	Progeny mean ± SE
T_1	14−4	85	17.3	7.4	0.563	27.7 ± 6.9	9.3 ± 1.3	17 .0 ± 6.0
	14−4	75	3.5	18.7	0.540	12.5 ± 5.3	41.0 ± 7.2	33.5 ± 6.0
	14−4	67	1.3	20.3	0.545	1.3 ± 0.9	69.7 ± 37.8	51 ± 37.7
T_2	24−4	85	26.7	0.2	0.721	0.0 ± 0.0	20.0 ± 0.0	0.0 ± 0.0
	24−4	75	4.0	16.9	0.629	16.0 ± 6.8	43.0 ± 23.1	39.0 ± 28.7
	24−4	67	1.6	19.3	0.590	108.0 ± 4.0	71.0 ± 16.0	159.0 ± 20.0
T_3	17−7	85	22.0	3.8	0.417	0.0 ± 0.0	25.3 ± 3.4	5.3 ± 3.4
	17−7	75	3.8	17.8	0.451	36.5 ± 16.5	20.0 ± 17.0	36.5 ± 0.5
	17−7	67	1.4	19.8	0.452	3.3 ± 1.3	32.3 ± 4.5	15.7 ± 3.7

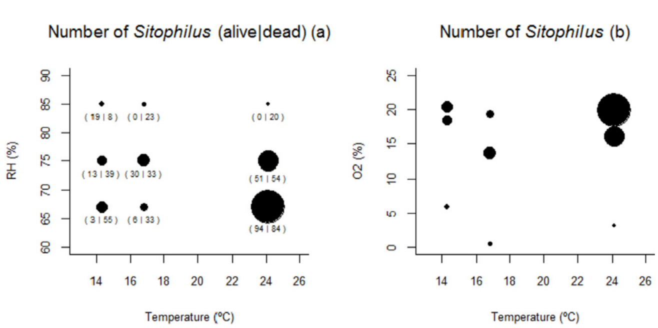

Fig. 5. (a) - Population growth of *Sitophilus zeamais,* for each pair of temperature (°C) and relative humidity (RH, %) conditions. The area of each circle is proportional to the sum S. alive + S. dead, while the values below each circle correspond to alive adults (left) and dead adults (right). (b) - Population growth of *Sitophilus zeamais* for each pair of temperature (°C) and oxygen content (%). The area of each circle is proportional to the sum alive adults + dead adults.

under hermetic conditions, corresponding to the storage time at on-farm facilities. The flours were obtained by milling the rice subjected to storage. In these tests, the viscoelastic parameters G' (storage or elastic modulus) and G'' (loss or viscous modulus) of the rice pastes, are measured. The viscoelastic behaviour of the gel pasts was considered as an indicator for the technological aptitude of each rice.

Fig. 6ab shows the gelling point (when G′ crosses over G″) of 10% (w/w) pastes of Japonica and Indica rice flour heated from 20 °C to 90 °C, at the inflexion point of the graph (around 85 °C) under the three different RHs. At the Japonica rice paste, there is a slight decrease in the gelling point temperature with the increase of RH: hermetic storage at 67%RH and control started gelling at about 87 °C; hermetic storage under 75% and 85% RH, started gelling at 85° and 82 °C, respectively. For the Indica rice pastes, the results were reversed: the control and hermetic storage at 85% RH started gelling at 85 °C and both hermetic conditions of 67% and 75% RH,

Table 6
Comparison of progeny across storage environmental conditions. The groups based on means were obtained with a post-hoc Tukey HSD test, while the groups based on ranks were based on a multiple comparison with Kruskal-Wallis test using the Fisher's least significant difference method. The critical value of the Studentized Range based on a Tukey's distribution with parameters 9 and 45, at 5% significance, is 4.606. The Fisher's least significant difference method is based on a t-Student distribution with 45 degrees of freedom. Treatments with the same letter are not significantly different at a 5% significance.

Treatment		Progeny	
Temp (ºC)_ time (months)	RH (%)	mean ± SE (adults per vial)	rank of adults per vial
24_4	67	158.0 ± 23.3^{a}	50.4^{a}
24_4	75	84.7 ± 31.7^{b}	37.5^{b}
17_7	75	43.5 ± 10.0^{bc}	37.8^{ab}
14_4	67	38.3 ± 15.6^{bc}	31.7^{bc}
14_4	75	32.4 ± 4.6^{bc}	34.1^{bc}
17_7	67	19.7 ± 7.5^{c}	24.9^{cd}
14_4	85	8.5 ± 4.7^{c}	15.9^{de}
17_7	85	3.3 ± 1.9^{c}	14.0^{de}
24_4	85	0.0 ± 0.0^{c}	7.0^{e}

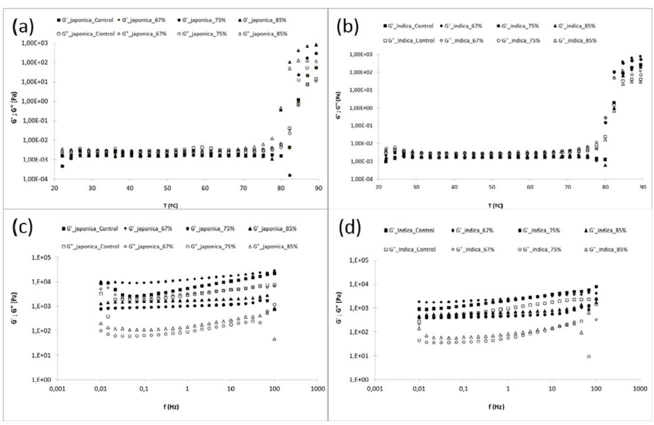

Fig. 6. (a)–(b) - Starch gelatinization of the rice paste. Heating from 20 °C to 90 °C at 2 °C/min (G′ elastic modulus; G″ viscous modulus). (a) - Japonica Rice; (b) - Indica Rice. (c)–(d) - Mechanical spectra at 20 °C (G′- elastic modulus; G″- viscous modulus). (c) - Japonica Rice; (d) - Indica Rice.

started gelling at 82 °C.

Fig. 6cd shows the mechanical spectra of the rice pastes by variety and relative humidity conditions. The paste obtained from rice stored at lower RH values, i.e., 67%, is slightly more structured than rice stored at higher humidity and control, i.e. the values of the viscoelastic parameters G′ and G″ are slightly higher for the pastes from rice stored under lower RH conditions, showing a better structured paste (Nunes et al., 2006).

4. Discussion

During the present study, *S. zeamais* showed distinct behaviour under low oxygen contents: no progeny was found under 3.8% O_2 content, but under 0.2% of O_2 the progeny registered an average of 5.3 adults. The response of stored-products insects to changes in O_2 and CO_2 contents differs within different species. For the majority of stored-product insects, the lower the oxygen concentration, the shorter the exposure time necessary to produce the complete kill. Navarro (1978) considered that this rule, on the oxygen deficit theory, has a classical exception with the *Sitophilus* spp. This species has been identified as amongst the most tolerant stored product insects to CO_2 rich atmospheres (Annis, 1987). Fleurat-Lessard and Le Torc'h (1986) observed that few immature stages of *S. granarius* were susceptible the treatment of 1% O_2 under 10° to 12 °C and the exposure periods of 3 weeks were necessary to kill adults. Other authors reported that the development of insects can be prevented when O_2 is blow 3% in the hermetic storage container within 30 days (Moreno-Martinez et al., 2000; Adhikarinayake et al., 2006). In Portugal, a 40t silo with polished rice under controlled atmospheres of 87.5% CO_2 and 2.6% O_2, during 10 days, two *S. zeamais* adults were alive, one in the sample nearest CO_2 entrance (Carvalho et al., 2012a). Njoroge et al. (2017) registered that acoustic activity rates dropped down of adult *Sitophilus oryzae* as oxygen fell below 5% and mortality was observed at 2% levels. Donahaye et al. (2001) demonstrated that exposure to O_2 levels of 3% and 5%, imparted a metabolic stress on adults and hypoxia at 2–5% O_2. Other authors reported controversial results. For instance Conyers and Bell (2007) mentioned that an increase of CO_2 to 10 or 20%, reducing O_2 to 5%, was sufficient to eliminate the emergence of *S. granarius* at 20 °C, but a few individuals emerged at 25 °C.

Modified atmosphere was naturally produced inside the hermetic bag under 85% RH, despite average temperature. Our results showed the importance of the relative humidity, average temperature and time as factors for the modification of the atmosphere content of paddy rice, hermetically stored. The modification of the air composition changes with the increase of the relative humidity, temperature, and storage time. Water activity decreases with storage time. Calderon and Navarro (1980) considered that when CO_2 is added to low O_2 atmospheres, there is a synergistic effect, from the significant interaction between the concentrations of these two gases. Carbon dioxide contents above 35% by volume of air were reported, by several authors, to be lethal to insects (Banks, 1983ab; Annis and van Graver, 1986; Fleurat-Lessard, 1990; Fleurat-Lessard and Le Torc'h, 1991), which was not attained in our experiments. Similar experiments using superbags, GrainPro® SuperGrainbag® Farm™, to store paddy rice during six months, were made in Mozambique, a tropical country (Guenha et al., 2014). The average temperature was 24 °C and moisture content 12%. At end of the trials, comparing traditional storage (jute bags) with hermetic storage results, there was a decrease of 96% of insect population on paddy stored hermetically. The gas content was not measured. Donahaye et al. (2001) considered that the influence of temperature on insect respiration, in tropical countries, showed that the O_2 intake by insects is very intensive and O_2 reduction may be rapid. Conversely in temperate regions, insects' metabolism is slower and insect control may not be achieved. In our experiments it was the influence of relative humidity, together with temperature and time of storage that influenced the O_2 and CO_2 content.

The modification of gas content had impact on the rheology of the rice pastes. The increase on respiration rate reduced the viscoelastic functions and changed the starch gelatinization point of Indica and Japonica rice, showing an impact on rice quality affecting the starch molecules. The increase on respiration rate, as an indirect measure of insects or grain physiological activity, registered for higher values of RH, reduced the viscoelastic functions and changed the starch gelatinization point of Indica and Japonica rice. Both insects and grain respiration affect starch, reducing the length of the starch macromolecule by hydrolysis, accessed through a glucose monomer, having a negative impact on the quality of the rice (Lagarrigue and Alvarez, 2001). Storing paddy hermetically at low relative humidity, did not change atmospheric content and maintained the viscoelastic functions of the rice pastes. Indica variety started gelling between 82 °C and 84 °C whatever the RH at storage. Japonica variety showed different behaviour: with the increase of the relative humidity the temperature of the gelling decreased.

At Portuguese conditions, using hermetic storage to store paddy rice at 67% RH, might not change the air composition, but avoid grain activity and maintain rice quality.

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